In the Claims:

Please amendment the claims as set forth in the listing of claims below.

Listing of Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently Amended) A method for ~~preparing~~ ascertaining the presence of fetal nucleated red blood cells (fetal NRBCs) ~~present in maternal peripheral blood~~ for prenatal genetic investigation,
comprising the steps of:

a.) mixing peripheral maternal blood and tissue culture medium to form a non-physiological tissue culture mixture having the following characteristics:

pH	6.4-6.6	
osmolarity	300-330	mOsm
Na^+	150-170	mmol/l
K^+	4.5-5.5	mmol/l
Cl^-	100-115	mmol/l
Ca^{++}	1.00-2.50	mmol/l
glucose	400-500	mg/dl
lactate	10-20	mg/dl

b) transferring the non-physiological tissue culture mixture obtained in step a) into a cell separation device, followed by introducing into said separation device a liquid having a density higher than maternal blood and containing a red blood cells (RBCs) aggregating agent,

c) in discontinuous density gradient, subjecting the separation device to centrifugal

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force to isolate a low density cell fraction having a lower density than the liquid introduced in step b) and wherein said low density cell fraction comprises fetal NRBCs;

d) collecting said low density cell fraction; and

e) ascertaining the presence of said fetal NRBCs in said low density cell fraction.

2. (Cancelled)

3. (Previously Presented) The method of claim 1 in which the non-physiological mixture obtained in step a) has the following characteristics:

pH	6.5	
osmolarity	320	mOsm
Na^+	165	mmol/l
K^+	5.35	mmol/l
Cl^-	110	mmol/l
Ca^{++}	1.25	mmol/l
glucose	500	mg/dl
lactate	10	mg/dl.

4. (Original) The method of claim 1 in which the RBCs aggregating agent of step b) is Ficoll.

5. (Original) The method of claim 1 in which the density of the liquid introduced in the separation device by the step b) is 1.068 g/ml.

6. (Previously Presented) The method of claim 1 in which the separation device used in step b), comprises an elongated chamber, whose cross section decreases from the base towards the top, at least a first channel one end of which opens into said chamber

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near said base and the other end is connected to a pressurized liquid source, and a second channel one end of which opens into the elongated chamber at the device top while the other end opens at the exterior of the device, said device further comprising at least one additional channel, one end of which opens at a middle level of said chamber height and the other end opens at the exterior of the device.

7. (Cancelled)

8. (Previously Presented) The method of claim 1, further comprising counting said fetal NRBCs.

9. (Previously Presented) The method of claim 1, in which the separation device has a base and a top and containing an elongated chamber whose cross section decreases from the base towards the top of the device which contains at least a first channel, one end of which opens on the inside of the chamber near the base and the other end connectable to a pressurized liquid source and a second channel whose end opens in the chamber at a level corresponding to the device top, wherein there is at least a third channel in the device, one end of which opens at an intermediate level of the chamber length, and the other end opens outwards from the device and wherein near the base a flow deflector is provided to disperse evenly through the entire cross section of the inside chamber of the incoming fluid arriving from the first channel.

10. (Previously Presented) The method of claim 1, wherein the isolation of said low density cell fraction in step c) is performed in one separation device.

11. (Currently Amended) Method for separating nucleated red blood cells (NRBCs) from maternal blood comprising:
providing peripheral maternal blood comprising nucleated red blood cells (NRBCs) and monocytes and lymphocytes having overlapping density distribution profiles;
causing the density of said NRBCs to decrease and the cell density of said monocytes

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and lymphocytes to increase by transferring said maternal blood into a non-physiological liquid comprising non-physiological tissue culture medium to create a non-physiological tissue culture mixture, wherein said non-physiological tissue culture mixture has a pH of 6.4 to 6.6; and

causing ~~substantial~~ a separation of said NRBCs from said monocytes and lymphocytes by subjecting said non-physiological tissue culture mixture to centrifugation in a discontinuous density gradient, wherein said NRBCs are present in a low density cell fraction.

12. (Previously Presented) The method of claim 11, wherein said tissue culture mixture has an osmolarity of 300-330 mOsm.

13. (Canceled)

14. (Previously Presented) The method of claim 11, wherein a liquid having a density higher than maternal blood and containing red blood cells (RBCs) aggregating agent is added immediately after transferring said maternal blood into said non-physiological liquid comprising non-physiological tissue culture.

15. (Previously Presented) The method of claim 14, wherein said agent is Ficoll.

16. (Previously Presented) The method of claim 11, wherein isolation of said low density cell fraction comprising said NRBCs is performed in a single separation device.

17. (Previously Presented) The method of claim 11, wherein, after said separation, said low density cell fraction comprising said NRBCs is transferred into a physiological tissue culture medium and the presence of fetal NRBCs is ascertained.

18. (Previously Presented) The method of claim 17, wherein the presence of said fetal NRBCs is ascertained by the presence of ε-chain hemoglobin and/or by FISH.

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19. (Previously Presented) The method of claim 18, wherein the presence of ε-chain hemoglobin is ascertained by anti-ε chain hemoglobin antibodies.

20. (Previously Presented) The method of claim 1, wherein isolation of said low density cell fraction can be accomplished in a single separation step.

21. (Previously Presented) The method of claim 11, wherein said NRBCs and monocytes and lymphocytes can be separated in a single separation step.

22. (Canceled)

23. (Canceled)

24. (Canceled)

25.   (Currently Amended) A method for separating nucleated red blood cells (NRBCs) from maternal blood comprising:
providing peripheral maternal blood comprising nucleated red blood cells (NRBCs) and lymphocytes and monocytes having overlapping density distribution profiles;
causing the density of said NRBCs to decrease and the cell density of said lymphocytes and monocytes to increase by mixing said maternal blood with a liquid comprising tissue culture medium unbuffered with a buffer that maintains a physiological pH to create a non-physiological tissue culture mixture; and
causing ~~substantial~~ a separation of a low density cell fraction comprising said NRBCs by subjecting said non-physiological tissue culture mixture to centrifugation in a discontinuous density gradient.

26. (Currently Amended) The method of claim 11, wherein ~~not more than 13000~~ 13767 or less nucleated cells of said low density cell fraction have to be analyzed for reliable

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non-invasive prenatal genetic investigation.

27. (Currently Amended) The method of claim 27, wherein ~~not more than 13000~~ 13767 or less nucleated cells of said low density cell fraction have to be analyzed for reliable non-invasive prenatal genetic investigation.

28. (Previously Presented) The method of claim 25, wherein isolation of said low density cell fraction comprising said NRBCs is performed in a single separation step.

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